FORM PTO-1390 (REV. 9-2001) — U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 02-314

U.S. APPLICATION NO (If known, see 37 CFR 1.5): 10/089486

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GR00/00028	September 27, 2000	September 29, 1999

TITLE OF INVENTION: CRYOPRESERVED AMNIOTIC HUMAN CELLS FOR FUTURE THERAPEUTIC, DIAGNOSTIC, GENETIC AND OTHER USES

APPLICANT(S) FOR DO/EO/US: SPYROS TSAKAS

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371
3. [X] This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below
4. [X] The US has been elected by the expiration of 19 months from the priority date (Article 31)
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is attached hereto (required only if not communicated by the International Bureau)
   b. [ ] has been communicated by the International Bureau
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2))
   a [ ] is attached hereto
   b. [ ] has been previously submitted under 35 U.S.C. 154(d)(4)
7. [ ] Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a [ ] are attached hereto (required only if not communicated by the International Bureau)
   b. [ ] have been communicated by the International Bureau
   c [ ] have not been made, however, the time limit for making such amendments has NOT expired
   d [ ] have not been made and will not be made
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3))
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4))
10. [ ] An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5))

Items 11 to 20 below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [ ] A FIRST preliminary amendment.
14. [ ] A SECOND or SUBSEQUENT preliminary amendment.
15. [ ] A substitute specification.
16. [ ] A change of power of attorney and/or address letter
17. [ ] A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. [ ] A second copy of the published international application under 35 U.S.C. 154(d)(4)
19. [ ] A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4)
20. [ ] Other items or information

I hereby certify that this correspondence is being deposited with the United States Postal Service as Express Mail in an envelope addressed to: U.S. Patent and Trademark Office, P.O. Box 2327, Arlington, VA 22202 on March 27, 2002.

Nicole Motzer

Nicole Motzer

March 27, 2002

Date of Signature

EXPRESS MAIL NO.: EL398539395US

U.S. APPLICATION NO (If known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
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21. [XX] The following fees are submitted	CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1040.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO . . **$890.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$740.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$710.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 890.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ --	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	29 - 20 =	9	x **$18.00**	$ 162.00	
Independent claims	2 - 3 =	-	x **$84.00**	$ --	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$280.00**	$ 280.00	
TOTAL OF ABOVE CALCULATIONS =				$1,332.00	
[XX] Applicant claims small entity status See 37 CFR 1.27 The fees indicated above are reduced by 1/2			+	$ 666.00	
SUBTOTAL =				$ 666.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$ --	
TOTAL NATIONAL FEE =				$ 666.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)) The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **$40.00** per property +				$ --	
TOTAL FEES ENCLOSED =				$ 666.00	
				Amount to be refunded:	$
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a. [X] A check in the amount of $ 666.00 to cover the above fees is enclosed

b. ☐ Please charge my Deposit Account No _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-0184 . A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

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29,999
REGISTRATION NUMBER

TITLE : Cryopreserved amniotic human cells for future therapeutic, diagnostic, genetic and others uses.

DESCRIPTION

Technical field of the invention

The invention refers to the field of biotechnology and more particularly to the field of cryobiology. The invention applies to genetic diagnosis and through this, to genetic therapies of human diseases or life theater accidents by use of already cryopreserved human amniotic cells.

State of the art

Within the amniotic fluid which surrounds the embryo there exist amniotic cells. These cells are biological elements (material) that exist outside of the body and of the development of the embryo. These amniotic cells originate from embryonic cells which are driven away from the embryo during pregnancy, since the early embryonic stages and are moving freely inside the amniotic fluid that surrounds the embryo, where they remain during pregnancy.

The amniotic cells are non-differentiated (primal) cells and are genetically identical with all other cells, differentiated or not, of the embryonic body from which they come from. The amniotic cells can easily be grown and multiplied in cell cultures.

Until now, the amniotic cells are lost in the environment together with the amniotic fluids at birth and they cannot be used for the embryo's benefit in its future life, which means after birth. Therefore, all humans loose at birth a unique and indispensable genetic material forever.

There is no way, under natural conditions, for the amniotic cells to be preserved in a living condition after birth and after the loss of amniotic fluid.

Nowadays, the only known use of amniotic cells is taking place before birth, during pregnancy and is taking place for clearly prenatal testing via amniocentesis.

More specifically, amniotic cells are taken together with amniotic fluid during pregnancy via amniocentesis for the diagnosis of biochemical, cellular, and chromosomal abnormalities of the embryo. During this procedure (Modern Genetics, F.J. Ayala & J.A. Kiger Jr. ed. Benjiamin, Cummings, p.p. 722-724), a sample of 10-15ml amniotic fluid that surrounds the embryo is taken by using a surgical syringe between the 14 and 16th week of pregnancy. Amniotic cells may also be taken during earlier stages of pregnancy by other means.

Then, the amniotic cells that exist in the amniotic fluid are separated by centrifugation and on them chromosomal number and aberrations are viewed. Besides, other biochemical tests are also performed on the amniotic fluid to see if some known genetic diseases out of the approximately 5000 existing ones are genetically determined in the embryo. Upon the results of this test depends the embryos' life, i.e. the interruption or not of pregnancy.

In the case that the amniotic sample is lost or in cases where more or new material is needed, it is required that a second amniocentesis takes place, if this is allowed by the pregnancy stage.

Object of the invention

The object of the present invention are the amniotic cells, that is cells which exist in the amniotic fluid surrounding the embryo. According to the present invention these cells are isolated from their natural environment and preserved in deep-freezing in a number of samples. Their life span is thus extended longer than their natural life-cycle, for long periods of time, with the goal to multiply and use them in the future for diagnostic, therapeutic and other purposes for the after birth life of the embryo.

Object of the invention are amniotic cells which have been multiplied before their storage, or which have been multiplied after their storage, their thawing and their new cryo-preservation.

It is known that the human body in the different stages of its formation (i.e. embryonic development) and its development cannot be the object of an invention for which a patent may be granted. However, one element (cell) which is isolated from the human body, such as the amniotic cells in the case of the present invention that are isolated by themselves and are flowing freely into the amniotic liquid, with the goal of being destroyed at birth, may represent a patentable invention. This is so, even if the genetic make-up of said the element (the amniotic cell which, according to the invention, has been preserved in a viable-useful state longer than their natural life span) is the same with that of the natural element, that is with the amniotic cell that is part of the amniotic fluid at the time of birth.

Technical problem

The existing technical problem, that is solved by this invention is that nowadays, amniotic cells are not preserved in a viable - useful condition after birth, so that they be used in the after-birth life of the embryo and after the amniotic cells' natural destruction.

The amniotic cells that are coming from the amniotic fluid have, in comparison to other cells, the following advantage and uniqueness: they are cells that are primary, primal and less differentiated to a great extent, as well as genetically identical to all other cells (differentiated or not) of the embryo's body, from which they originate. Due to these properties the amniotic cells can be the basic material for the production by differentiation of nearly any future differentiated cell of the body, which (differentiated cell) they can substitute genetically, functionally and physiologically. It is thus possible that epidermal cells are produced from amniotic cells for use in plastic surgery.

Under certain conditions, amniotic cells have the prerequisites and the possibility to be differentiated into a number of the 200 existing different human body cells' categories.

Amniotic cells cannot be developed and differentiated by themselves; they have the potential though to be differentiated under specific control lab conditions that are constantly expanding and improving, into categories of differentiated cells that may be of future use to the embryo, that is in its after-birth life.

In the existing technical state of the art, it is not possible for diagnosis of new genetically determined diseases to take place on amniotic cells, besides those that are already known today, due to the non preservation of amniotic cells after birth. For genetic diseases that are already known to science, the only possible diagnosis on amniotic cells is prenatal diagnosis that takes place before birth. However, for diseases that will be known to science in the future, at a stage after the embryo's birth and at any age, it is no longer possible to use amniotic cells for diagnostic purposes in relation to those, because today these cells are lost during birth.

Besides, with the existing technical state of the art, it is not possible to appreciate the genetic predisposition for the development of diseases such as breast cancer, prostate cancer, nor is it possible to appreciate other genetically related genetic diseases like hypertension and heart diseases by using amniotic cells. The genetic cause of these diseases is still unknown, but this might be known in the future, after birth and during life of the human being, whose amniotic cells have been preserved according to the present invention. Thus, for the human being, whose amniotic cells have been preserved after birth, it will be possible at least to diagnose harmlessly genetic diseases.

Amniotic cells are unique cell material that cannot be replaced, which may serve not only for diagnostic purposes, but can also provide the basic cell material for gene and genetic therapy. Under the current state of the art, it is not possible to use amniotic cells for diagnosis or treatment of diseases, the treatment of which is still unknown during birth, or of day life accidents that may take place after birth, as well as of infectious diseases after birth. The reason for this is that following birth,

there do not exist any amniotic cells preserved somewhere for use after birth.

According to the present invention, amniotic cells that are preserved in a viable state after birth offer the possibility to diagnose new genetic diseases and to appreciate the genetic predisposal for the development of diseases, as well as to apply methods of gene or other genetic treatment in the case of genetic relative diseases, accidents and infectious diseases, during each person's life.

Amniotic cells according to the present invention can also be used to produce cells resistant to pathogens, viruses, bacteria, to be used for the creation of new cell lines, for the treatment of wounds, burns, for the addition of tissues for therapeutic or aesthetic reasons.

Another application of the present invention is that it offers the possibility to create healthy cells, tissue and organs from the amniotic cells that are stored and preserved in a viable state. These cells, tissue and organs can be used to replace non-healthy or malfunctioning similar ones and they can also be used to produce genetically identical copies - clones of the person they belong to.

These above mentioned new products, which may have been genetically modified or not, are genetically identical to corresponding cells, tissue and organs of their owner who is the recipient. Therefore, these are compatible with him, which means that when drafted, they have extremely few possibilities to be rejected. For instance, such cells are the muscular cells of cardiac valves, among others.

Due to their preservation according to the present invention, the stored amniotic cells even if contaminated by infection factors such as HIV virus, do have the possibility to be freed by the infection factors by means of specific culture condition. Therefore, they can be used as healthy amniotic cells for therapeutic and other uses thereafter.

Another application of the present invention is that due to the preservation of viable amniotic cells, we may establish a person's

genetic identity (f.e. DNA fingerprint) which may follow the person during his whole future life. The genetic identity may be established when the sample is being taken, or later, after thawing of the preserved cells. Following the existing state of the art, in cases were it is necessary to verify the genetic data of an infant in cases of erroneous information, unwanted adoption or unwanted paternity recognition, as well as of paternity matters for succession, it is necessary to take blood samples from both the father and the child for testing. Because of the present invention, such a new test will not be necessary because each human being may have a ready genetic identity and stored genetic material. The same application is possible also when it is not possible to take a genetic sample in the case of a deceased person, which is necessary for criminology.

Establishing the genetic identity of persons with the use of amniotic cells preserved according to the present invention offers the unique advantage of the creation of collective ready genetic data. This collective data is providing also the potential of unique genetic properties to be discovered and consequently to be used and exploited for research, diagnostic, therapeutic and genetic uses.

For example, while a human being was initially a HIV carrier, at a further stage of his life he is becoming virus-free and it is discovered that this is related to the possession of a specific genetic factor, which has been discovered to be the reason for the discard of the virus. By use of the collective genetic data assembled from the establishing of genetic identities it is possible to find out who else possesses the same genetic factor and this in turn may be used for the above purposes.

Another application of the present invention is that amniotic cells that are preserved according to the present invention for uses that will take place after birth, uses in respect to the donor or his next of kin, do own and preserve certain qualities due to the present invention. More specifically, they can be used to create differentiated human cells with much larger probability of success than any other cell category, because they are undifferentiated and genetically related to the recipients.

The stem cells, which are used today with the existing state of the art after being cryopreserved, are coming from the umbilical cord or from bones' marrow tissue. These cells have no relation to the amniotic fluid and what this includes, they are cells more differentiated in comparison to amniotic cells, and due to this differentiation it is relatively more difficult that they be differentiated in other cell categories. Besides, these stem cells also belong to tissues of the embryo, i.e. they are part to the development of the embryo, they differ morphologically and physiologically from amniotic cells and they are used in particular for cancer therapy.

Amniotic cells which are preserved viable and useful for future uses at a time after human birth and which are the object of the present invention, possess due to the present invention certain properties: because they are primal and undifferentiated, they can be used to produce differentiated cell lines of the human body with much greater probabilities of success comparatively to other existing cell categories; they can also be used for detection and therapy of diseases that appear after human birth, i.e. cancer, for genetic engineering and therapy and for curing infectious diseases and accidents that may happen during human life.

One way of taking amniotic cells and separating them from the amniotic fluid for their preservation according to this invention is the following:

During pregnancy, a quantity of amniotic fluid is taken with the amniocentesis process, or in any other way.

1. The amount of amniotic fluid varies between 20 to 80 ml for the needs of the present methodology.

2. The amniotic fluid is properly centrifuged and the amniotic cells are taken from the pellet.

3. The original amniotic cells are cultured by using one of the existing cultures substrates, such as MEM medium.

[redacted]

4. Cryoprotective compounds such as glycerine, DMSO or polyethylene glucols in consentrations between 5 to 50% are added to the amniotic cells.

5. A line of five vial from the start culture is produced and five more lines with 5 vials each, from the rest of the culture, are created by seeding. Thus, we have the first line, which consists of 5 vials on which we are working performing the necessary tests and five more lines, each with five vials, for the deep-freezing process.

6. The temperature of the samples (vials) starts falling via a very sensitive computer controlled system at a pace not more than one degree Celsius (1°C) per min.

7. Ice nucleation process starts by dropping microcrystals of ice so as to create ice from outside the cells, and so as to avoid the creation of crystals within the cells, which will destroy them.

8. Finally the temperature starts dropping faster up to the 150 degrees Celsius below zero (-150°C) and usually the vials are kept in liquid nitrogen (-196°C) where the total of the 25 vials are kept, see original paper of Pentz and Horler. (Pentz S. and Horler H. J. Med. Genet. 1980, Dec. 17 (6) : 472-5).

9. There is a number of thawing processes that are known and that can be applied. In general, the thawing process is nearly a reverse course of the freezing process, were the main consideration is again to avoid ice crystal formation within the cells and the osmotical removal of the cryoprotective compounds of glycerine or DMSO. It has recently been mentioned that even simple exposure to environmental temperature may be equally useful.

The amniotic cells can be prepared for preservation also in other ways, for example using freezing, dehydrating and closing the amniotic cells in polymers or liposomes etc., and then deep-freezing storage takes place as above or at much higher temperatures than that of liquid nitrogen (-196°C).

The amniotic cells' mixture that is being cryopreserved as described above can differ as to the degree of amniotic cells' concentration: from nearly only amniotic cells, up to pure amniotic fluid.

Freezing processes similar to the above described, are used in early human embryos' deep-freezing during the process of artificial insemination. These embryos are much more sensitive to cryopreservation than amniotic cells, because as they are differentiated and developed they have larger probabilities of being destroyed.

In the near future, new, better, simpler and less expensive methods of cryopreservation of amniotic cell will apply.

The present invention provides the possibility to preserve amniotic cells of human embryos for a long time after birth.

One further object of the present invention is the amniotic cells that can be produced during the life-span of a human being, in other words after birth. These amniotic cells can be produced today using adults' mammary gland cells or even epidermal cells.
The experiments at Roslin Institute in Scotland have proven that with the nuclear transfer technology from a mammary gland cell to a sheep ovary from where the nucleous had been removed, it is possible to create a viable embryo-clone, Dolly. With an approximately similar technology human embryo-clones may also be produced.
It is permitted and legitimately and scientifically acceptable nowadays to produce human clone embryos and to store them up to 14 days of age. It is probable that this time limit will be extended in the future to longer than 14 days, for experimental and medical, genetic.

After a human embryo is created in the above way, the said embryos contain inside them in their early stages freely-floating amniotic cells. These early amniotic cells are ignored today for the existing level of state

of the art, that is they are lost after the termination of life of the embryo-clone. There is no way to preserve these amniotic cells in a viable condition under natural circumstances, after the loss of the embryo.

The amniotic cells that are taken from the embryo-clone early in its life are necessary for the case that no amniotic cells, that are originating from the amniotic fluid, have been preserved viable after birth, that is in the case that a human looses the opportunity offered by the present invention, in its first object as this is described above.

The specific object of the present invention wishes to solve this new technical problem. That is, each individual is given the opportunity to acquire his/her own amniotic cells, that are genetically identical to him/her and are identical to the amniotic cells that he had lost at birth, because his amniotic cells that came from amniotic fluid had not been stored after birth.

The amniotic cells that exist free inside the internal part of the early embryo-clone, which can be produced as above for each individual, can be taken easily by using, for example, a micropipete injection under a reverse microscope used for microinjection.

After being collected in the above or in another way, the clone's amniotic cells can then be multiplied in cells culture media and then cryopreserved as mentioned earlier for the amniotic cells coming from the amniotic fluid.

The process of embryo-clone production should not appear strange to us since already nowadays a huge number of embryos are produced, stored and then abandoned for the needs of artificial insemination.

The present object of the invention, that is the cryopreservation of amniotic cells coming from the embryo-clone at its early stages, has the same applications as the first object of the invention, that is as the cryopreserved amniotic cells that come from the amniotic fluid and are taken during pregnancy.

With its further object, the present invention is offering to living persons the advantage offered to embryos according to its first object, that is the

possibility to preserve their amniotic cells so that these be useful after birth.

Therefore, the present invention offers to all living human beings, to those that are in embryonic stage as well as to those that will be born in the future, the possibility to use their own amniotic cells and to use all the benefits and advantages which genetics, biotechnology and medicine can offer based on their cryopreserved amniotic cells.

A further object of the present invention, is also every biological material which will be produced directly or indirectly from the cryopreserved amniotic cells described above; further object of the present invention is also any biological product or by-product which will be the outcome of reproduction, multiplication or extraction under the same or a modified form by the use of the above described cryopreserved amniotic cells. The said biological material, biological product or by-product will possess the same genetic properties and applications as the cryopreserved amniotic cells described above.

CLAIMS

1. Amniotic cells that are taken from amniotic fluid which surrounds the human embryo at the early stages of its development and that are preserved in a viable and useful state out of their natural environment, after birth and after their natural destruction, by way of their deep-freezing (cryopreservation). These amniotic cells may be :

a) multiplied before their cryopreservation, or

b) cryopreserved without previously having been multipled, or

c) multiplied after thawing following their cryopreservation and are again cryopreserved for long preservation.

2. Amniotic cells that are taken from human body cells, through the creation from those of embryo – clone, younger or older of the age of 14 days. These amniotic cells are isolated from their natural environment and are preserved, through their cryopreservation, in a viable – useful state after the end of the life of the embryo – clone. These amniotic cells may be:

d) multiplied before their cryopreservation, or

e) cryopreserved without previously having been multipled, or

f) multiplied after thawing following their cryopreservation and are again cryopreserved for long preservation.

3. Compositions which contain a) a number of amniotic cells according to each of claims 1 and 2 in different concentrations, starting from pure amniotic cells up to pure amniotic fluid, as well as b) compounds which make the amniotic cells able for long cryopreservation, such compounds being glycerin, or dimethylsulfoxide (DMSO), or polyethylene glycols (PEG's).

4. Amniotic cells according to each of claims 1, 2 and 3, which at a point in time posterior to their natural loss and destruction are used at any time :

[redacted]

a) for genetic, diagnostic and therapeutic reasons.

b) for the application on them of genetic identification.

c) for establishing any kind of genetic identities data (such as DNA fingerprint) for diagnostic, therapeutic, social, legal, succession, criminololical and other purposes.

5. Amniotic cells according to claim 4, that offer a collective ready sample of genetic material and offer the possibility to identify unique genetic properties and their use and exploitation for research, diagnostic, therapeutic, genetic and commercial purposes.

6. Amniotic cells according to claims 1 to 5 that are used for:

a) on-time diagnosis and therapy of genetic diseases or genetic predisposition to diseases or functional failures,

b) gene therapy, or for their differentiation in cells, tissues and organs for substituting the ones which suffer failures,

c) therapy from diseases or accidents, for the creation of cell lines resistant to pathogens, viruses, bacteria, for the creation of new cell lines, for the healing of wounds, burns, for the addition of tissues for therapeutic or cosmetic purposes.

7. Amniotic cells according to claims 1, 2, 4 and 6 that are useful for the creation of differentiated cell lines categories, from the approximately two hundred (200) existing ones in the human body.

8. Amniotic cells according to claims 1, 2, 6 and 7, that are used through cultures to produce tissues and in the future to produce organs or even genetic clones of their owner.

9. Biological compound, which will be directly or indirectly produced from amniotic cells according to claims 1, 2, 5, 6, 7 and 8, as well as any biological product or by-product which will be produced from the use of such cryopreserved amniotic cells through their reproduction or

multiplication, under the same or under different form, and which possesses the same genetic properties and applications as them.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 April 2001 (05.04.2001)

PCT

(10) International Publication Number
WO 01/23532 A1

(51) International Patent Classification⁷: C12N 5/08, A01N 1/02, A61K 35/50

(21) International Application Number: PCT/GR00/00028

(22) International Filing Date:
27 September 2000 (27.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
990100331 29 September 1999 (29.09.1999) GR

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(81) Designated States *(national)*: AE, AU, BG, BR, CA, CN, CR, CZ, EE, GD, GE, HR, HU, ID, IL, IN, IS, JP, KE, KP, KR, LK, LT, LV, MA, MK, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TR, UA, US, YU, ZA.

(84) Designated States *(regional)*: Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette

(54) Title: CRYOPRESERVED AMNIOTIC HUMAN CELLS FOR FUTURE THERAPEUTIC, DIAGNOSTIC, GENETIC AND OTHERS USES

(57) Abstract: This invention belongs to the field of biotechnology, cryobiology and human therapeutics. Object of the invention are a) amniotic cells originating from amniotic fluid that surrounds the embryo in its early development stages, b) amniotic cells that at early embryonic stage are floating within the human embryo-clone, c) every biological material which will be created directly or indirectly from the above amniotic cells, as well as every biological product or by-product, which will be the outcome of the use of the said cryopreserved amniotic cells, through their reproduction or multiplication under the same or any other modified form and which will possess the same genetic properties and applications. These amniotic cells are isolated from their natural environment and are care preserved in deep-freezing for very long periods of time after their own natural life-span, which is short within their natural environment, that is within the amniotic fluid and within the embryo-clone. The purpose is the genetic and medical use of these cells at a time posterior to their natural destruction. In this way, we succeed to use primal, undifferentiated and genetically identical cells of each human being for diagnosis and therapy of genetic or non-genetic diseases, malfunctions and accidents, where these are known nowadays or shall be applied in the future.

WO 01/23532 A1

Cryo

Practitioner's Docket No. 02-314 ***PATENT***

COMBINED DECLARATION AND POWER OF ATTORNEY

(ORIGINAL, DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL, DIVISIONAL, CONTINUATION, OR C-I-P)

As a below named inventor, I hereby declare that:

TYPE OF DECLARATION

This declaration is of the following type:

(check one applicable item below)

- ☐ original.
- ☐ design.
- ☐ supplemental.

NOTE: If the declaration is for an International Application being filed as a divisional, continuation or continuation-in-part application, do not check next item; check appropriate one of last three items.

- ☒ national stage of PCT.

NOTE: If one of the following 3 items apply, then complete and also attach ADDED PAGES FOR DIVISIONAL, CONTINUATION OR C-I-P.

NOTE: See 37 C.F.R. § 1.63(d) (continued prosecution application) for use of a prior nonprovisional application declaration in the continuation or divisional application being filed on behalf of the same or fewer of the inventors named in the prior application.

- ☐ divisional.
- ☐ continuation.

NOTE: Where an application discloses and claims subject matter not disclosed in the prior application, or a continuation or divisional application names an inventor not named in the prior application, a continuation-in-part application must be filed under 37 C.F.R. § 1.53(b) (application filing requirements — nonprovisional application).

- ☐ continuation-in-part (C-I-P).

INVENTORSHIP IDENTIFICATION

WARNING: *If the inventors are each not the inventors of all the claims, an explanation of the facts, including the ownership of all the claims at the time the last claimed invention was made, should be submitted.*

My residence, post office address and citizenship are as stated below, next to my name. I believe that I am the original, first and sole inventor *(if only one name is listed below)* or an original, first and joint inventor *(if plural names are listed below)* of the subject matter that is claimed, and for which a patent is sought on the invention entitled:

TITLE OF INVENTION

CRYOPRESERVED AMNIOTIC HUMAN CELLS FOR FUTURE THERAPEUTIC, DIAGNOSTIC, GENETIC AND OTHER USES,

SPECIFICATION IDENTIFICATION

the specification of which:

(complete (a), (b), or (c))

(a) ☒ is attached hereto.

NOTE: "The following combinations of information supplied in an oath or declaration filed on the application filing date with a specification are acceptable as minimums for identifying a specification and compliance with any one of the items below will be accepted as complying with the identification requirement of 37 CFR 1.63:

"(1) name of inventor(s), and reference to an attached specification which is both attached to the oath or declaration at the time of execution and submitted with the oath or declaration on filing;

"(2) name of inventor(s), and attorney docket number which was on the specification as filed; or

"(3) name of inventor(s), and title which was on the specification as filed."

Notice of July 13, 1995 (1177 O.G. 60).

(b) ☐ was filed on _____, as ☐ Serial No. 0 /_____ or ☐ _____ and was amended on _____ *(if applicable).*

NOTE: Amendments filed after the original papers are deposited with the PTO that contain new matter are not accorded a filing date by being referred to in the declaration. Accordingly, the amendments involved are those filed with the application papers or, in the case of a supplemental declaration, are those amendments claiming matter not encompassed in the original statement of invention or claims. See 37 C.F.R. § 1.67.

NOTE: "The following combinations of information supplied in an oath or declaration filed after the filing date are acceptable as minimums for identifying a specification and compliance with any one of the items below will be accepted as complying with the identification requirement of 37 CFR 1.63:

"(A) application number (consisting of the series code and the serial number, e.g., 08/123,456);

"(B) serial number and filing date;

"(C) attorney docket number which was on the specification as filed;

"(D) title which was on the specification as filed and reference to an attached specification which is both attached to the oath or declaration at the time of execution and submitted with the oath or declaration; or

"(E) title which was on the specification as filed and accompanied by a cover letter accurately identifying the application for which it was intended by either the application number (consisting of the series code and the serial number, e.g., 08/123,456), or serial number and filing date. Absent any statement(s) to the contrary, it will be presumed that the application filed in the PTO is the application which the inventor(s) executed by signing the oath or declaration."

M.P.E.P. § 601.01(a), 7th Ed.

(c) ☒ was described and claimed in PCT International Application No. PCT/GR00/00028, filed on 27 September 2000 and as amended under PCT Article 19 on _____ *(if any).*

SUPPLEMENTAL DECLARATION (37 C.F.R. § 1.67(b))

(complete the following where a supplemental declaration is being submitted)

☐ I hereby declare that the subject matter of the

☐ attached amendment

☐ amendment filed on _____

was part of my/our invention and was invented before the filing date of the original application, above-identified, for such invention.

ACKNOWLEDGEMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information, which is material to patentability as defined in 37, Code of Federal Regulations, § 1.56,

(also check the following items, if desired)

☒ and which is material to the examination of this application, namely, information where there is a substantial likelihood that a reasonable Examiner would consider it important in deciding whether to allow the application to issue as a patent, and

☐ in compliance with this duty, there is attached an information disclosure statement, in accordance with 37 C.F.R. § 1.98.

PRIORITY CLAIM (35 U.S.C. §§ 119(a)–(d))

NOTE: *"The claim to priority need be in no special form and may be made by the attorney or agent if the foreign application is referred to in the oath or declaration as required by § 1.63. The claim for priority and the certified copy of the foreign application specified in 35 U.S.C. 119(b) must be filed in the case of an interference (§ 1.630), when necessary to overcome the date of a reference relied upon by the examiner, when specifically required by the examiner, and in all other situations, before the patent is granted. If the claim for priority or the certified copy of the foreign application is filed after the date the issue fee is paid, it must be accompanied by a petition requesting entry and by the fee set forth in § 1.17(i). If the certified copy is not in the English language, a translation need not be filed except in the case of interference; or when necessary to overcome the date of a reference relied upon by the examiner; or when specifically required by the examiner, in which event an English language translation must be filed together with a statement that the translation of the certified copy is accurate." 37 C.F.R. § 1.55(a).*

I hereby claim foreign priority benefits under Title 35, United States Code, §§ 119(a)–(d) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

(complete (d) or (e))

(d) ☐ no such applications have been filed.

(e) ☒ such applications have been filed as follows.

NOTE: *Where item (c) is entered above and the International Application which designated the U.S. itself claimed priority check item (e), enter the details below and make the priority claim.*

PRIOR FOREIGN/PCT APPLICATION(S) FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS APPLICATION AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. § 119(a)-(d)

COUNTRY (OR INDICATE IF PCT)	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 37 USC 119
GREECE	990100331	29.09.1999	☒ YES NO ☐
			☐ YES NO ☐
			☐ YES NO ☐
			☐ YES NO ☐
			☐ YES NO ☐

CLAIM FOR BENEFIT OF PRIOR U.S. PROVISIONAL APPLICATION(S)
(34 U.S.C. § 119(e))

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

PROVISIONAL APPLICATION NUMBER	FILING DATE
_____ /_____	_____
_____ /_____	_____
_____ /_____	_____

CLAIM FOR BENEFIT OF EARLIER US/PCT APPLICATION(S) UNDER 35 U.S.C. § 120

☐ The claim for the benefit of any such applications are set forth in the attached ADDED PAGES TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR DIVISIONAL, CONTINUATION OR CONTINUATION-IN PART (C-I-P) APPLICATION.

ALL FOREIGN APPLICATION(S), *IF ANY*, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

_____

_____

_____

NOTE: If the application filed more than 12 months from the filing date of this application is a PCT filing forming the basis for this application entering the United States as (1) the national stage, or (2) a continuation, divisional, or continuation-in-part, then also complete ADDED PAGES TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR DIVISIONAL, CONTINUATION OR C-I-P APPLICATION for benefit of the prior U.S. or PCT application(s) under 35 U.S.C. § 120.

POWER OF ATTORNEY

I hereby appoint the following practitioner(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

(list name and registration number)

Robert H. Bachman (19,374), Gregory P. LaPointe (28,395), Barry L. Kelmachter (29,999), George A. Coury (34,309), and Jeffrey R. Ambroziak (47,387), all of Bachman & LaPointe, P.C., 900 Chapel Street, Suite 1201, New Haven, CT 06510-2802

(check the following item, if applicable)

☒ I hereby appoint the practitioner(s) associated with the Customer Number provided below to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

☐ Attached, as part of this declaration and power of attorney, is the authorization of the above-named practitioner(s) to accept and follow instructions from my representative(s).

SEND CORRESPONDENCE TO

☒ Address

Bachman & LaPointe, P.C.
900 Chapel Street, Suite 1201
New Haven, CT 06510-2802

☐ Customer Number _____

DIRECT TELEPHONE CALLS TO:
(Name and telephone number)

Barry L. Kelmachter
(203) 777-6628 - ext. 114

DECLARATION

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

SIGNATURE(S)

NOTE: Carefully indicate the family (or last) name, as it should appear on the filing receipt and all other documents.

NOTE: Each inventor must be identified by full name, including the family name, and at least one given name without abbreviation together with any other given name or initial, and by his/her residence, post office address and country of citizenship. 37 CFR § 1.63(a)(3).

NOTE: Inventors may execute separate declarations/oaths provided each declaration/oath sets forth all the inventors. Section 1.63(a)(3) requires that a declaration/oath, inter alia, identify each inventor and prohibits the execution of separate declarations/oaths which each sets forth only the name of the executing inventor. 62 Fed. Reg. 53,131, 53,142, October 10, 1997.

Full name of sole or first inventor

SPYROS (GIVEN NAME) C. (MIDDLE INITIAL OR NAME) TSAKAS (FAMILY OR LAST NAME)

Inventor's signature Spyros C. Tsakos

Date March 27, 2002 **Country of Citizenship** GREECE

Residence 2 Mesologiou Sq., GR-116 34 Athens, GREECE GRX

Post Office Address (Same As Above)

Full name of second joint inventor, if any

(GIVEN NAME) (MIDDLE INITIAL OR NAME) (FAMILY OR LAST NAME)

Inventor's signature

Date **Country of Citizenship**

Residence

Post Office Address

Full name of third joint inventor, if any

(GIVEN NAME) (MIDDLE INITIAL OR NAME) (FAMILY OR LAST NAME)

Inventor's signature

Date **Country of Citizenship**

Residence

Post Office Address

(check proper box(es) for any of the following added page(s) that form a part of this declaration)

☐ **Signature** for fourth and subsequent joint inventors. *Number of pages added* _____.

* * *

☐ **Signature** by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor. *Number of pages added* _____.

* * *

☐ **Signature** for inventor who refuses to sign or cannot be reached by person authorized under 37 CFR 1.47. *Number of pages added* _____

* * *

☐ Added page for **signature** by one joint inventor on behalf of deceased inventor(s) where legal representative cannot be appointed in time. (37 CFR 1.47)

* * *

☐ Added pages to combined declaration and power of attorney for divisional, continuation, or continuation-in-part (C-I-P) application.

☐ Number of pages added _____

* * *

☐ Authorization of practitioner(s) to accept and follow instructions from representative.

* * *

(if no further pages form a part of this Declaration, then end this Declaration with this page and check the following item)

XX This declaration ends with this page.